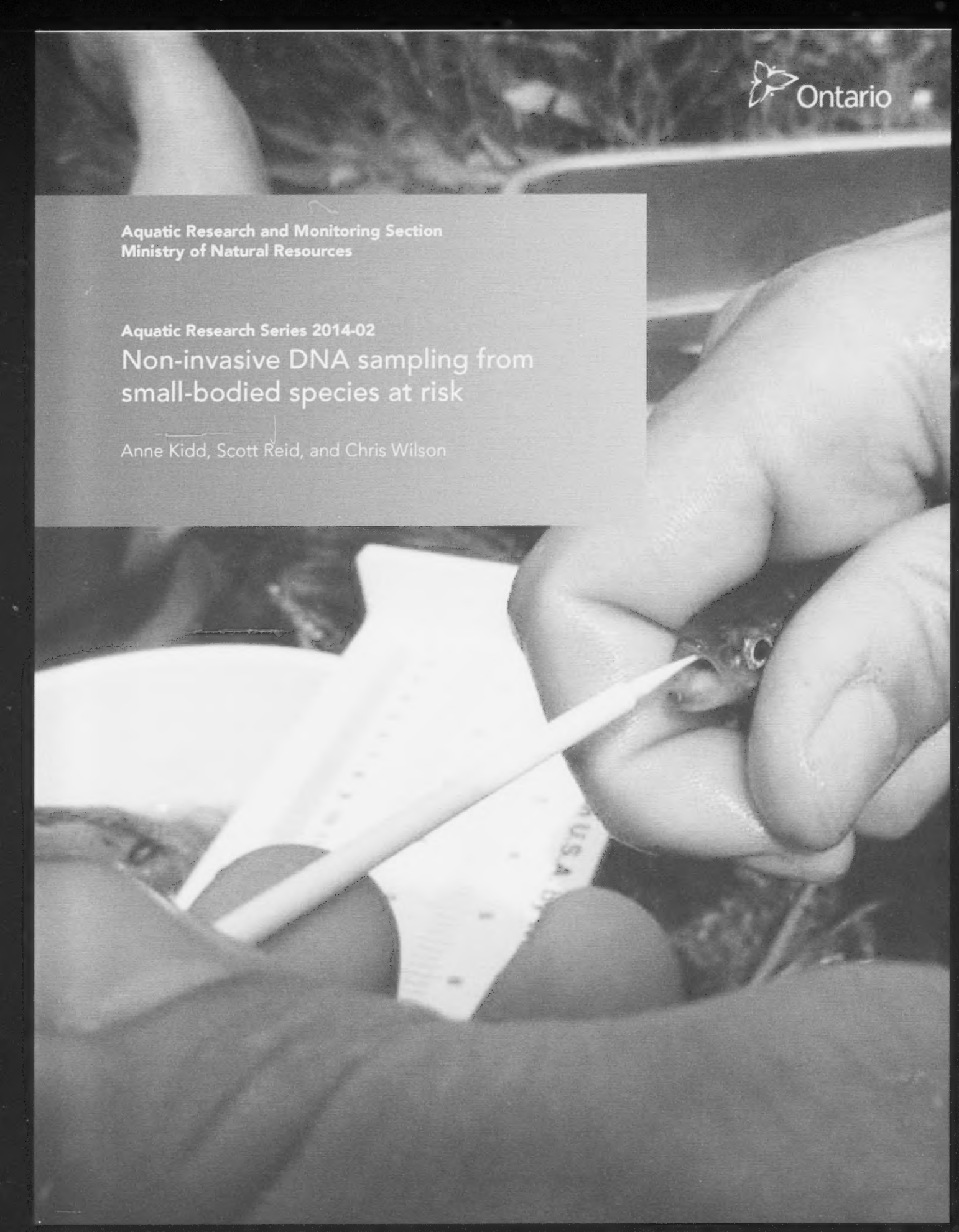

Ontario

Aquatic Research and Monitoring Section
Ministry of Natural Resources

Aquatic Research Series 2014-02

Non-invasive DNA sampling from small-bodied species at risk

Anne Kidd, Scott Reid, and Chris Wilson

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Cover photo: Non-lethal DNA collection from redside dace.
Photo credit: Anne Kidd

Cette publication hautement spécialisée Échantillonnage non invasif de l'ADN de petites espèces en péril n'est disponible qu'en anglais en vertu du Règlement 411/97, qui en exempte l'application de la Loi sur les services en français. Pour obtenir de l'aide en français, veuillez communiquer avec le ministère des Richesses naturelles au informationarms@ontario.ca.

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Abstract

This study assessed the effectiveness of using non-invasive mouth swabs for obtaining DNA from small-bodied aquatic species at risk. The method was tested on wild populations of redside dace (*Clinostomus elongatus*) and channel darter (*Percina copelandi*) by taking swab samples as well as non-lethal fin clips and testing DNA yield and quality. In addition, young-of-year brook trout (*Salvelinus fontinalis*) were sampled and held under controlled conditions for six weeks to assess the effects of handling, swab sampling, and fin clipping on their survival and growth. The results showed that swab sampling worked well for obtaining DNA without harming fish, and provides a method for obtaining genetic data from small fish without jeopardizing their survival.

Résumé

Cette étude a évalué l'efficacité de l'utilisation d'écouvillons buccaux non invasifs pour obtenir l'ADN de petites espèces aquatiques en péril. La méthode a été testée sur des populations sauvages de méné long (*Clinostomus elongatus*) et de fouille-roche gris (*Percina copelandi*) en prélevant des échantillons buccaux ainsi que des échantillons non létaux de nageoire, et en testant le rendement et la qualité de l'ADN. En outre, des ombles de fontaine (*Salvelinus fontinalis*) de l'année ont été échantillonnés et maintenus dans des conditions contrôlées durant six semaines afin d'évaluer les effets de la manipulation, du prélèvement d'échantillons buccaux et de nageoire sur leur survie et leur croissance. Les résultats ont montré que le prélèvement d'écouvillons a bien fonctionné pour obtenir l'ADN sans nuire au poisson, et que cette méthode permet d'obtenir des données génétiques de petits poissons sans mettre leur survie en péril.

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Introduction

Genetic information is widely used for the management and conservation of exploited and endangered species. Genetic-based research has been used to address a variety of issues such as species identification, unresolved evolutionary relationships, delineation of management units, and identification of suitable donor populations for re-introductions (Champagne et al. 2008, Jelks et al. 2008, Howes et al. 2009). Collecting tissue for DNA analysis, however, can be invasive or require lethal sampling. Given the small population sizes of many endangered fishes, sampling methods that cause increased mortality, stress, or other non-lethal effects (e.g. reduced growth) are undesirable.

Advances in genetic technology such as the polymerase chain reaction (PCR) have greatly reduced the amount of tissue required for genetic analysis and fin clips, scales, and blood are now widely used to obtain DNA. For small species or early life-stages, however, fin clipping and lethal sampling are commonly used because of tissue or volume limitations. Compared to larger fish, a standard clip can remove a significant fin portion (e.g. half their tail fin). Some studies have raised concerns that fin clipping can lower survival, affect growth, and alter behaviour (Hansen 1988, Le Vin et al. 2011), although other studies suggest these risks to be minimal (Pratt and Fox 2002, Champagne et al. 2008). Validation of non-invasive sampling methods will help minimize adverse effects on species and populations, and help researchers obtain collection permits for endangered species.

Non-invasive collection methods such as body, buccal (mouth), and viscera swabs have been shown to be effective for sampling DNA from amphibians, fish, and mussels (Pidancier et al. 2003, Campanella and Smalley 2006, Henley et al. 2006). While buccal swabs have successfully collected DNA from large-bodied fishes (Campanella and Smalley 2006, Livia et al. 2006), it has only recently been validated for small-bodied fishes (Reid et al. 2012). In this study we tested whether buccal swabs from small-bodied fishes-at-risk could provide DNA of similar quantity and quality for genotyping as taking tissue samples such as fin clips. Comparisons were based on field DNA collections from two imperiled Ontario fishes: channel darter (*Percina copelandi*) and redside dace (*Clinostomus elongatus*). At the Ontario Ministry of Natural Resources (OMNR) Codrington fisheries research facility, we also investigated the short-term effects of swab and fin clip sampling on young-of-year (YOY) brook trout (*Salvelinus fontinalis*).

Methods

Channel darter and redside dace

Channel darter and redside dace were collected from two tributaries of Lake Ontario (Salmon River and Duffins Creek) with a Smith-Root™ backpack electrofisher. Captured fish were held in tubs of river water until sampled and released. Swab samples (Figure 1)were collected from redside dace using a 6 mm diameter sterile brush (QIAGEN Buccal collection brushes; Figure 2a), and from channel darter using a 2 mm diameter non-sterile brush (Microbrush® International; Figure 2b). In both cases, brushes were gently rubbed inside the mouths of individual fish for five seconds (full sampling details are listed in Appendix 1). Care was taken not to damage gills or gill arches.

The brush ends of each swab were placed in individual 2 mL screw-cap cryovials with 1 mL of lysis buffer (1x TNES-urea buffer; Appendix 2). Caudal fin clips were collected from the same individuals and stored in 2 mL vials with 95% ethanol. Figure 2c shows labeled cryovials with a swab sample and fin clip from the same individual.

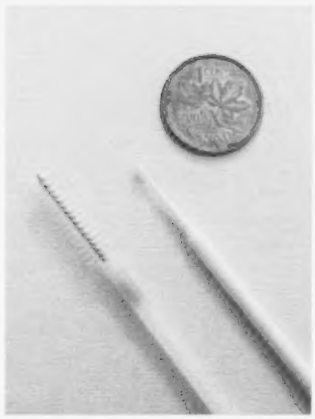

Figure 1: Large and small sampling brushes (QIAGEN buccal swab [left] and Microbrush®) for DNA swab sampling, with a penny for scale.

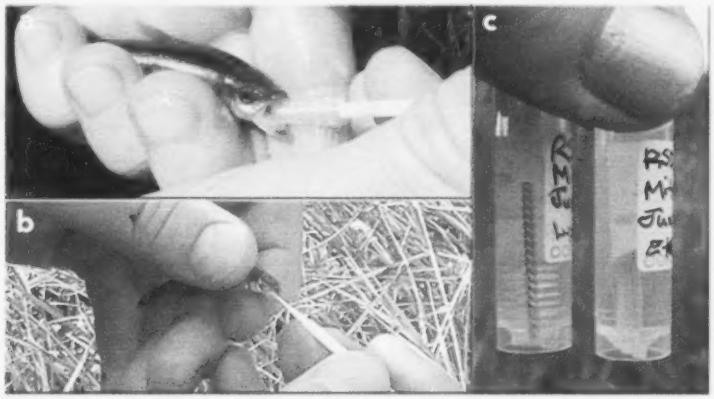

Figure 2: Taking swab samples from a redside dace (a) and channel darter (b). Swab the fish's cheeks and tongue gently; care should be taken to avoid damaging the fish's gills or gill arches. If handling the fish is expected to cause significant stress, sedating or anaesthetizing the fish may be advisable. (c) Swab sample and fin clip from a redside dace placed in labeled cryovials with suitable preservatives (lysis buffer and ethanol, respectively).

DNA was extracted from swab samples using the QIAGEN Buccal cell core kit. DNA from fin clips were extracted using a simple lysis method for fish tissue (Wilson et al. 2007). DNA quantity and quality were assessed by gel electrophoresis on 1.5% agarose gels alongside molecular mass ladders. Working solutions of DNA template were made using a 1:30 dilution of stock DNA with ddH_2O for fin clips, and a 1:15 dilution for buccal swabs.

Channel darter samples were genotyped at ten microsatellite and redside dace samples were genotyped at seven microsatellite loci each (Gabel et al. 2008, Dutton et al. 2008, Pitcher et al. 2009). Primer sequences, allele size ranges, annealing temperatures (TA) as well as PCR cocktail and conditions are detailed in Reid et al. (2012). Amplified DNA was visualized on an AB 3730 sequencer, and genotypes were scored using GeneMapper 3.1 (Applied Biosystems). Each individual's fin clip and swab genotypes were compared for congruency of allele sizes and peak height (quantity and reliability of PCR product).

Brook trout

At the Codrington fisheries research facility, YOY brook trout (surplus from rearing experiments) were randomly assigned to one of three experimental treatments, or a control group. YOY were anaesthetized with MS-222, then measured for weight (g) and total length (mm), and sampled by either (1) taking an oral swab with a fine microbrush as described above, (2) swab sampling a 5 mm x 5 mm area along their left side below the dorsal fin,

or (3) clipping a 5 mm x 5 mm portion of the upper lobe of the caudal fin. The control treatment involved the same handling, weighing and measuring, but without any DNA sampling. Four replicates of 12 YOY were used for each treatment. Trout were revived in cold, well-oxygenated water and held in early rearing tanks for eight weeks. At the end of the experiment in October, brook trout were euthanized and re-measured.

Results

We collected 20 channel darter (total lengths: 51 to 65 mm) and 19 redside dace (total lengths: 71 to 93 mm). No mortalities were observed during sampling. The channel darter sampling site was re-sampled two weeks after initial sampling. Recaptures of previously-sampled fish showed marked tail regeneration (Figure 3).

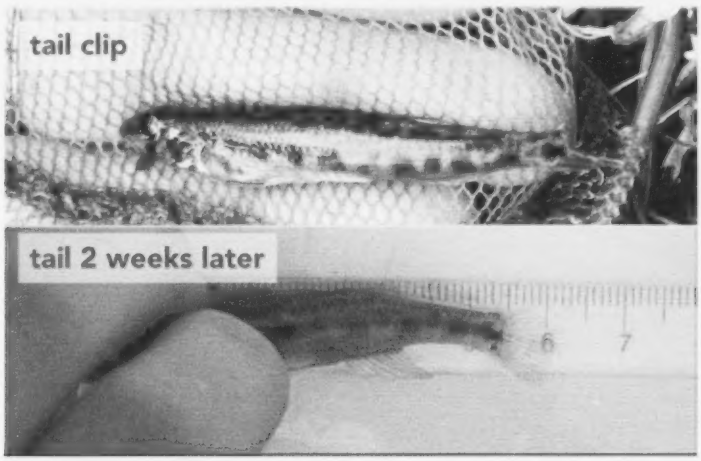

Figure 3: Non-lethal fin clip sampling of an adult channel darter (top image) and evidence of fin regeneration two weeks later (bottom).

DNA extracted from swab samples was of similar quantity and quality as fin clips (Figure 4). Overall, microsatellite amplification from the swabs was equally effective as those from fin clips (Figure 5). For both species, there was excellent agreement between genotypes amplified from swab and fin clip samples (Figure 5). Only three of two hundred channel darter comparisons did not match (0.015%), and all redside dace sample pairs yielded identical genotypes between swab and fin samples. The genotyping error rates (1.5% and 0% respectively), calculated as described by Broquet and Petit (2004), were comparable or lower than swabs of buccal cells and saliva from larger bodied fishes, and body mucus from small and large fishes (Livia et al. 2006, Le Vin et al. 2011), as well as from physical tissue samples (Broquet and Petit 2004).

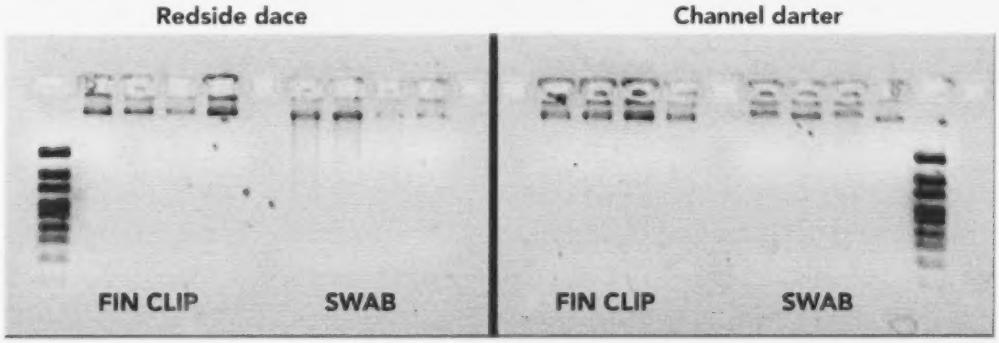

Figure 4: Extracted DNA from redside dace (left) and channel darter (right), showing comparative DNA yields from fin clip and swab samples. Size standards on the sides of the gels are also used as mass (yield) indicators to quantify how much DNA is obtained.

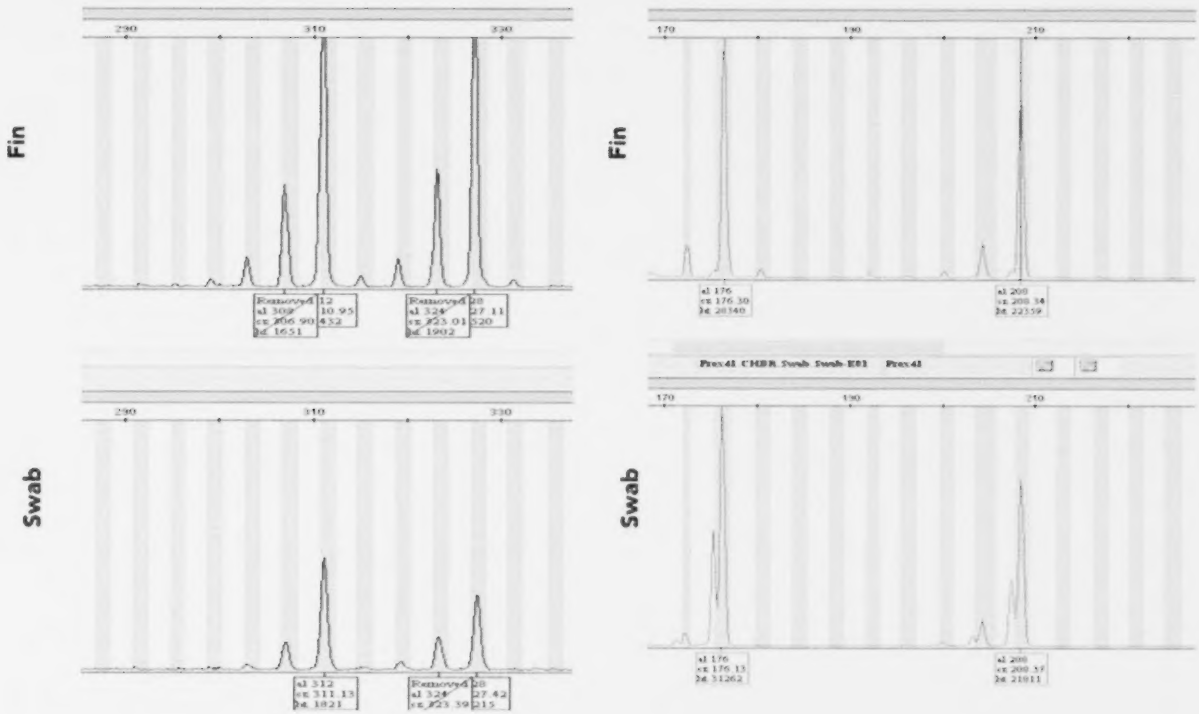

Figure 5: Amplified microsatellite data from fin clip and swab samples from redside dace (left) and channel darter (right), showing genotype data from the same individuals based on DNA from a fin clip (top) versus buccal swabs (bottom). The numbers below each peak indicate the raw and interpreted allele sizes as well as their intensity (peak height) when visualized on a ABI 3730 automated sequencer.

YOY brook trout sampled using swabs, microbrushes and caudal fin clips showed no adverse effects in comparison with YOY in the control group. Over the eight week period, mean total length increased by 11 to 12 mm, and mean weight increased by 1.5 to 1.6 g (Table 1). No significant differences in length or weight among treatments were detected (ANOVA, $p > 0.8$). Additionally, there were no mortalities or observable signs of stress (e.g. abnormal swimming behaviour). At the end of the experiment, clipped caudal fins were almost completely regenerated (Figure 6). As a caveat, carrying out the experiment in a benign environment with unlimited food and no predators may have eliminated potential differences among treatments that might otherwise have been observed under more natural circumstances.

Table 1. Mean (± standard error) total length (mm) and weight (g) of YOY brook trout sampled with swabs and fin clips at the time of sampling (August 2011) and after eight weeks (October 2011).

	August 19, 2011		October 14, 2011	
Treatment	Length	Weight	Length	Weight
Control	63.7 ± 0.7	2.19 ± 0.08	75.9 ± 0.8	3.76 ± 0.10
Buccal Swab	62.9 ± 0.7	2.13 ± 0.07	75.1 ± 0.8	3.67 ± 0.12
Body Swab	63.5 ± 0.6	2.17 ± 0.06	74.8 ± 0.7	3.62 ± 0.10
Fin Clip	64.7 ± 0.6	2.19 ± 0.08	76.2 ± 0.7	3.79 ± 0.09

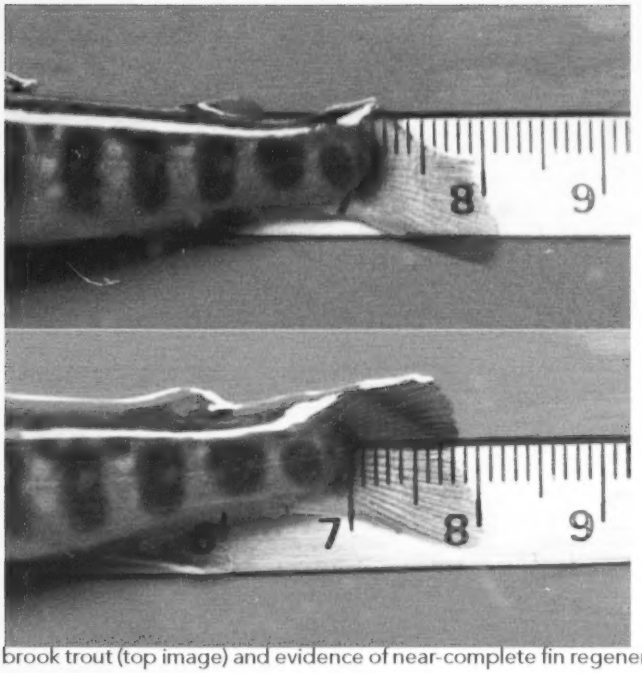

Figure 6. Fin clipped YOY brook trout (top image) and evidence of near-complete fin regeneration after eight weeks (bottom).

Conclusions

Our results show that swab sampling was highly effective for obtaining genetic samples without causing harm to small-bodied fishes. Swab samples were not only as effective for obtaining DNA as the fin clip samples, but yielded sufficient DNA of similar quality and amounts for obtaining high-quality genotype data. Despite the small size of the sampled fish (50 to 95 mm long) and swabs (2 and 6 mm diameter), the amount of DNA obtained is sufficient for an informative genetic study without the need for fin clips or other invasive sampling. This should be particularly advantageous, as fin clipping can result not only in stress to fish, but may also affect their behavior and survival (Weber and Wahle 1969, Mears and Hatch 1976, Hansen 1988, Le Vin et al 2011). Avoiding this physical and metabolic stress may be a significant consideration for sampling small-bodied and/or stress-sensitive species, as opposed to larger fish or life stages for which a fin clip would be less energetically costly.

One limitation of swab sampling is that after fish are released, it is not known which individuals within a population have been sampled. Target sample sizes for genetic studies typically range from 20 to 50 individuals per population: collecting a sufficient sample from low density populations may require multiple sample visits. In these cases, marking previously sampled individuals is required, and fin clipping might still be the most effective option for obtaining DNA samples. For these populations, significant fin regeneration can be expected within several weeks, and would enable discrimination between previously sampled and unsampled individuals.

In conclusion, using buccal swabs for DNA sampling is an effective, minimally intrusive method that will facilitate the collection of genetic data that can provide valuable information for the effective conservation and rehabilitation of small imperiled fish, without jeopardizing their survival. In some cases, using this sampling method must be balanced with needs for physically marking fish to avoid resampling. Overall, however, buccal swab sampling can be an effective tool for sampling small and/or sensitive fish species without physical damage, bioenergetics costs, or the risk of other sublethal impacts. For aquatic species at risk in particular, this sampling method provides an opportunity to obtain useful genetic information without negatively impacting the population of interest.

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Appendix 1: swab sampling for DNA (basic protocol)

The following protocol is limited to DNA sample collection, and assumes that captured fish will be live-released after sampling. Recommended sampling gear for the collection of fishes at risk can be found in:

C.B. Portt, G.A. Coker, N.E. Mandrak and D.L. Ming. 2008. Protocol for the detection of fish species at risk in Ontario Great Lakes Area (OGLA). Canadian Science Advisory Secretariat Research Document 2008/026. www.dfo-mpo.gc.ca/csas-sccs/publications/resdocs-docrech/2008/2008_026-eng.htm

Care should be taken to minimize handling stress of fish during sampling, as minimizing negative effects on sampled individuals is a primary goal of this protocol. Provincial (Endangered Species Act) and federal (Species at Risk Act) scientific collection permits are required for listed threatened and endangered species.

Things you'll need:

- swabs or microbrushes (appropriate for the size of the fish's mouth)
- small volume tubes for holding brush tips
- preservative solution for DNA samples (95% ethanol, lysis buffer, *RNA*later, or alternatives)
- fine scissors
- lab gloves (vinyl, latex, or nitrile)
- tube storage rack or box
- fine-tip permanent marker

Procedure

It is recommended that sample tubes are filled with preservative before field collections. Simply add 1.0 to 1.5 mL of preservative solution to individual tubes and reseal them. Do not overfill the tubes, as the swab or microbrush will add to the volume. This will save time in the field when handling live fish, and minimize loss of sample and solutions.

The advisability of sedating or anaesthetising fish prior to taking swab samples will depend on the species and life stage, its vulnerability to handling stress, and the expertise of the field crew. If necessary, sedate or anaesthetise the fish with an approved anaesthetic to minimize stress during handling. If using an anaesthetic is expected to increase physiological or handling stress or result in higher mortality, it may be preferable to not use an anaesthetic.

Using a fresh dry swab or brush, **gently** rub/wipe/brush the inside of the fish's mouth for 5 seconds, focusing on the cheeks and tongue. Be careful to not damage the gills or gill arches. Place the swab into a tube with 1.0 to 1.5 mL of preservative solution, and cut off enough of the swab's handle to be able to cap the tube. Close and **label** the tube, being sure to record location, date, and individual data. Tubes with DNA samples can be kept at room temperature or in a refrigerator. If it is expected that samples will sit for an extended period of time before DNA extraction, freezing at or below -20°C is advisable for long-term storage.

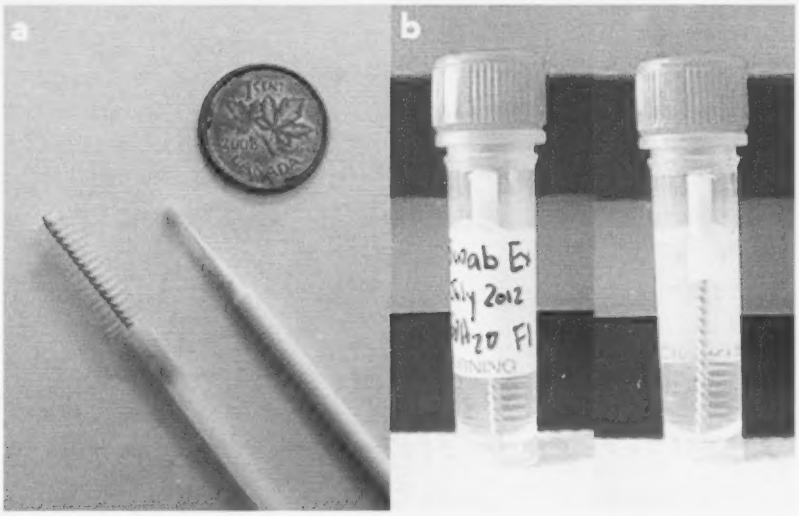

Figure 1: Example of suitable swabs or microbrushes and sample preservation a) Qiagen buccal swab and Lee Valley microbrush with a penny for scale b) Swab ends cut and stored in labeled 2 mL cryovials filled with preservative.

Figure 2: Examples of using a swab (a) and microbrush (b) that is suitable for the fish being sampled.

Appendix 2: Detailed list of materials and suggested suppliers

Item	Supplier	Catalog number
Preservatives		
95% Ethanol	Commercial Alcohols Ltd.	Ethyl Alcohol (95%)
RNAlater® preservative	Life Technologies	AM7020
	Qiagen	
		76104
Lysis buffer	Qiagen	158822 (10 preps)
	Gentra Puregene Buccal Cell Kit	158845 (100 preps)
		158867 (400 preps)
DNA lysis buffer (MNR lab kit)	MNR fish genetics lab, Trent U.	1x TNES-Urea buffer
Tubes/Vials		
Corning	Fisher or VWR	430917
Axygen	Fisher, VWR or Ultident	MCT-150-C (1.5 mL tubes)
Buccal swabs		
Buccal Swabs	Qiagen	As above
	Gentra Puregene Buccal Cell Kit	
Microbrushes	Lee Valley Tools Ltd.	Superfine – 1.1mm (83K09.21)
		Fine Ball – 2.0mm (83K09.22)
		Regular Ball – 2.8mm (83K09.23)
Other		
Tube Storage boxes	Fisher or VWR	various
Fine-tip permanent marker	Staples / Grand and Toy	Sharpie
Scissors		

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